



First record of *Macrobrachium grandimanus* (Randall, 1840) (Crustacea, Decapoda, Palaemonidae) from Guam, Micronesia

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Abstract

The Hawaiian River Shrimp, *Macrobrachium grandimanus* (Randall, 1840), is an amphidromous brackish water prawn that occurs in the Hawaiian Islands (type locality), Ryukyu Islands, Melanesia (Fiji, New Caledonia), and Polynesia (Tonga). Two genetically and morphologically differentiated lineages of this species are known, i.e., the Hawaiian and the Ryukyuan lineages. Here, we report a new record of this species from Guam, Micronesia. Morphological and mitochondrial DNA analyses revealed that the Guam population is closely related to the Ryukyu lineage.

Keywords

Amphidromous, Decapoda, DNA barcoding, haplotype network, mitochondrial DNA, Ryukyu Archipelago

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Introduction

The Hawaiian River Shrimp, *Macrobrachium grandimanus* (Randall, 1840), is a brackish-water prawn that is widely distributed in the Pacific Islands and has an amphidromous life history (Shokita 1985). *Macrobrachium grandimanus* is known to be distributed in the Hawaiian Islands (type locality), Ryukyu Islands, Melanesia (Fiji, New Caledonia), and Polynesia (Tonga) (Kubo 1940; Holthuis 1950; Maciolek and Yamada 1981; Shokita 1985; Short and Marquet 1998; Fig. 1). The Hawaii and Ryukyu populations are distinctly diverged based on genetic and morphology; since the type locality of the species is Hawaii, the Ryukyu population is considered a cryptic species (Holthuis 1950; Liu et al. 2007). However, the distributional range of each lineage remains

unknown because comparisons have not been performed in other regional populations. To resolve the taxonomic confusion of this species, it is necessary to compare different populations from various localities. Here, we report the first record of *M. grandimanus* from Guam, Micronesia, and reveal that the specimens are genetically and morphologically clustered with the population from the Ryukyu Islands.

Methods

Seven specimens of *M. grandimanus* were collected from the Hagåtña (Agana) River, Guam (Fig. 2), and 14 specimens from Ishigaki-jima Island, Ryukyu Islands, Japan,

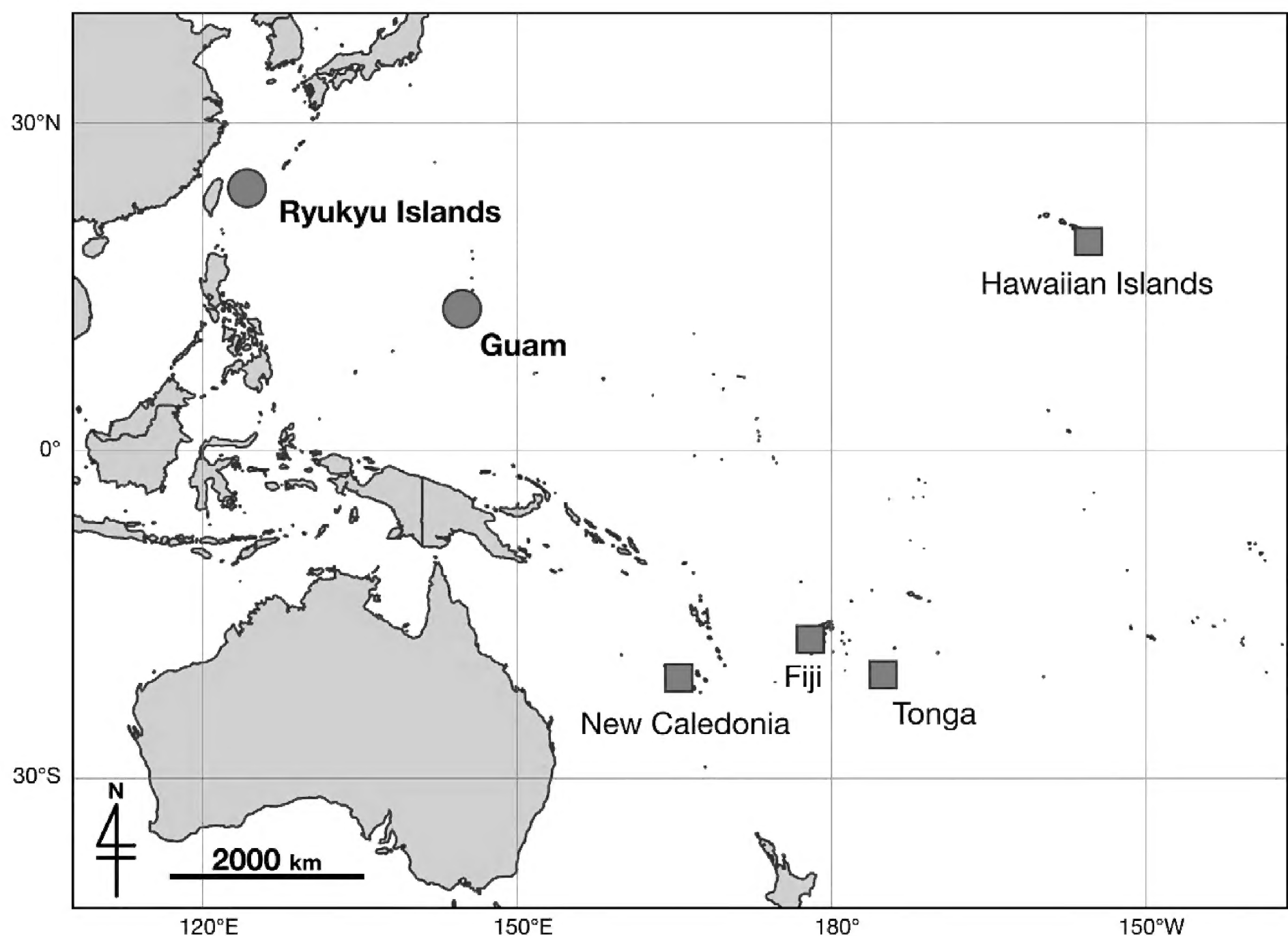


Figure 1. Distribution map of *Macrobrachium grandimanus*. Circles represent the collection sites of specimens used in this study, and squares represent distribution records based on literature records.

for genetic and morphological comparisons. Hand nets were used for collection. All samples were preserved in 70% ethanol. The examined specimens were deposited in the Zoological Collection of Kyoto University, Japan (KUZ).

Measurements were performed for postorbital carapace length (CL), and segment of the 1st to 3rd pereopods with digital calipers (CD67-S20PM, Mitutoyo, Japan) after preservation.

Total DNA was extracted from the 4th or 5th pleopod using a Genomic DNA Purification kit (Promega, USA). A fragment of the mitochondrial 16S rRNA gene was polymerase chain reaction (PCR)-amplified using the primer pair 16sA (5'-ACT TGA TAT ATA ATT AAA GGG CCG-3') and 16sB (5'-CTG GCG CCG GTC TGA ACT CAA ATC-3') (Wowor et al. 2009). The PCR amplification conditions were as follows: initial denaturation (94 °C, 2 min); 30 cycles of denaturation (94 °C, 30 s), annealing (52 °C, 30 s), and extension (72 °C, 60 s); and a single final extension (72 °C, 7 min). The PCR products were purified with Illustra ExoStar (GE Healthcare Japan, Japan) and sequenced on ABI 3130xl Genetic Analyzer (Applied Biosystems, USA) using amplification primer and the BigDye Terminator Cycle sequencing FS Ready Reaction Kit v. 3.1 (Applied Biosystems). The partial 16S rRNA sequences were deposited in the DDBJ/EMBL/GenBank (accession numbers:

LC530806–LC530812, LC569848–LC569861).

Sequences of the Hawaiian and Ryukyuan *M. grandimanus* specimens were obtained from the International Nucleotide Sequence Database and added to the following analysis (DQ194925 and DQ194926; Liu et al. 2007). Multiple alignments were performed using MAFFT (Kato and Standley 2013) on Unipro UGENE (Okonechnikov et al. 2012) for the obtained sequences. To visualize the relationships between local populations, the haplotype network was estimated using PopART (Leigh and Bryant 2015) based on the TCS algorithm (Clement et al. 2000).

Results

Order Decapoda

Family Palaemonidae Rafinesque, 1815

Genus *Macrobrachium* Spence Bate, 1868

Macrobrachium grandimanus (Randall, 1840)

Material examined. GUAM • Hagåtña River estuary, Hagåtña; 13.4775, 144.7545; 18.XI.2018; Makoto Sasazuka leg.; under a rock; 4♂, 3♀; CL 6.5–10.9 mm; KUZ Z3780; GenBank: LC530806–LC530812.

JAPAN • Toro River, Ishigaki-jima Island, Okinawa, Ryukyu Islands; 24.4647, 124.2499; 12.III.2020; Yusuke Fuke leg.; roots of mangrove; 7♂, 7♀; CL 6.0–13.7 mm;

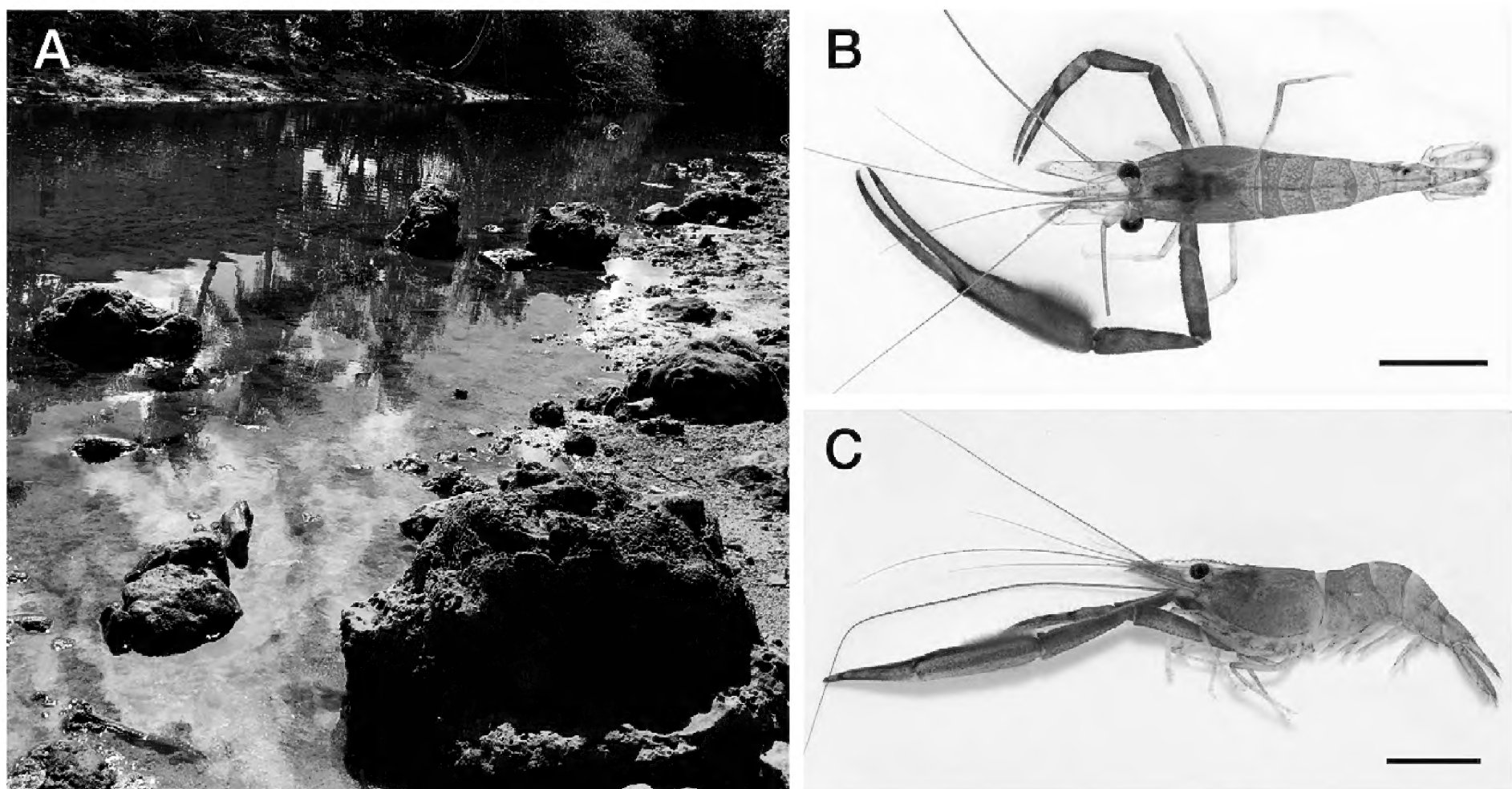


Figure 2. *Macrobrachium grandimanus* collected from Guam (male, CL 10.9 mm, KUZ Z3780) and its habitat. **A.** Habitat of *M. grandimanus* in Guam. **B.** Dorsal view. **C.** Lateral view. Scale bars: B, C = 10 mm.

KUZ Z3781; GenBank: LC569848–LC569861.

Identification. The specimens collected from Guam were identified as *M. grandimanus* based on morphological characters (Holthuis 1950; Cai and Jeng 2001). Rostrum straight, reaching end of third segment of antennular peduncle; dorsal margin with 13 or 14 teeth, including 3 or 4 on carapace posterior to orbital margin; ventral margin with 2–4 teeth ($n = 7$). In three adult males, first pereopod slender; finger 0.7–0.9 times shorter than palm; palm 0.3–0.4 times shorter than carpus; carpus 1.1–1.2 times longer than merus; merus 1.6–2.1 times longer than ischium. In one adult male, second pereopods unequal in shape and size; major second pereopod (left) robust, longer than total body length; tips of finger curved inwards, finger slightly shorter than palm; surface of palm with dense setae on last 2/3, palm 1.3 times longer than carpus; carpus 1.3 times longer than merus; merus 2.4 times longer than ischium; minor second pereopod (right) slender; finger 2.1 times longer than palm; palm 0.6 times shorter than carpus; carpus 1.2 times longer than merus; merus 1.7 times longer than ischium (Fig. 2). In two adult males, third pereopod slender; dactylus 0.3 times shorter than propodus; propodus 1.5–1.8 times longer than carpus; carpus 0.5–0.6 times shorter than merus; merus 2.1–2.3 times longer than ischium.

Genetic analysis. We obtained 441 bp of 16S rRNA of *M. grandimanus* mitochondrial DNA from the Guam population, the Ryukyu population, and including the Hawaii population obtained from the GenBank. Haplotype network analysis revealed two haplotypes from the Guam population and six haplotypes from the Ryukyu population; no haplotypes were shared among the three populations (Fig. 3). There were 14 or 15 base

substitutions (3.2–3.4%) between the Guam and Hawaii populations, and 1–4 base substitutions (0.2–0.9%) between the Guam and Ryukyu populations.

Discussion

Guam is the largest oceanic island in Micronesia, located at the southern end of the Mariana Islands in the western Pacific Ocean (Fig. 1). It is an unusual island in the Mariana Islands since there are abundant of inland water bodies. At least five species of atyid river shrimp, *Antecaridina lauensis* (Edmondson, 1935), *Atyoida pilipes* (Newport, 1847), *Caridina variabilis* (de Mazancourt, Marquet, Rogers & Keith, 2018), *C. typus* (Milne Edwards, 1837), and *Halocaridinides* sp., and four species of palaemonid river prawn, *Macrobrachium equidens* (Dana, 1852), *M. lar* (Fabricius, 1798), *M. rosenbergii* (De Man, 1879) (as introduced species), and *Palaemon debilis* (Dana, 1852), have been reported from Guam (Concepcion and Nelson 1999; Leberer and Cai 2003; Paulay et al. 2003; de Mazancourt et al. 2018). All of these species are amphidromous. Some fishes, crustaceans, and snails with amphidromous life histories are known to be dispersed by ocean currents during their larval stages (McDowall 2010; Anger 2013; Abdou et al. 2015). The planktonic larval duration of *C. typus*, *M. equidens*, *M. lar*, and *P. debilis* is >22, 36–53, 77–110, and 20–24 days, respectively (Ngoc-Ho 1976; Shokita 1977; Nakahara et al. 2007; Lal et al. 2014). *Macrobrachium grandimanus* is also an amphidromous species with small eggs, and its larvae reach decapodid-stage in 27–30 days in the Ryukyu population (Shokita 1985). The length of those larval duration would have been sufficient for those species to reach the oceanic island of Guam; however, for *M. grandimanus*, it is assumed to be

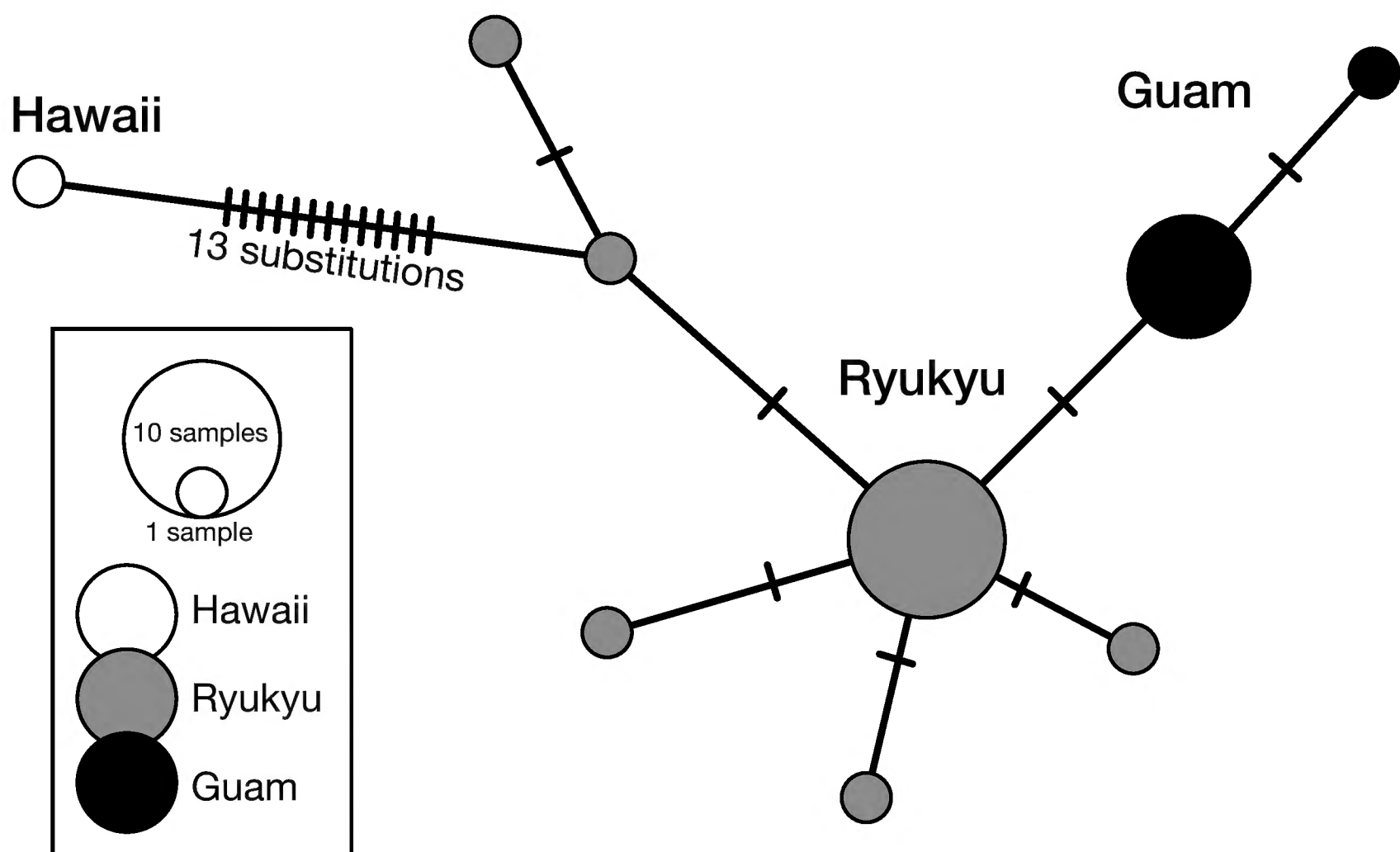


Figure 3. TCS haplotype network of *Macrobrachium grandimanus* based on 441 bp of 16S rRNA mitochondrial DNA. A black bar represents a single base substitution.

too short to facilitate frequent genetic exchange between Hawaii and Guam, and also between Hawaii and the Ryukyu Islands. That this species does not share a haplotype among the three populations may be due to the small sample size or the absence of stepping-stones (i.e., a staging post). To clarify the formation history of the Guam population, higher resolution genomic analysis and confirmation of the presence or absence of this species in the potential staging post, for example, the Philippines are necessary.

Holthuis (1950) highlighted a difference in the rostral formula between the Ryukyuan and Hawaiian *M. grandimanus* populations (11–15/3 vs. 14–17/4–5, respectively). Furthermore, Lui et al. (2007) conducted a mitochondrial DNA-based phylogenetic analysis of East Asian *Macrobrachium* species, including *M. grandimanus* populations from the Hawaii Island and Ryukyu Islands, and reported that this species has a remarkable regional differentiation. Since the type locality of this species is Hawaii, the Ryukyu population is considered to be a pseudocryptic species. The rostral formula in the present Ryukyu population is 12–14/3–5, and Guam population is 13–14/2–4, agreeing with the Ryukyu population. The haplotype network analysis based on mitochondrial DNA also showed close relationship between the Guam and Ryukyu populations (Fig. 3). If the Hawaii and Ryukyu populations are considered to be different species, this Guam population would be attributed to the same species represented by the Ryukyu population. These results are consistent with the direction of the North Equatorial Current and the Kuroshio Current (Chang et al. 2015). Short and Marquet (1998) reported four *M. grandimanus* individuals from New Caledonia

and described the rostral formula as 14–17/3–7, indicating that the New Caledonian population is similar to the Hawaiian population. In the future, it will be necessary to examine morphological traits other than the rostral formula and to make comparisons using genome-wide markers throughout the distribution area. Studies on the taxonomic organization and population development of *M. grandimanus* will provide important insights into the diversity formation of amphidromous migratory organisms in the western Pacific.

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Authors' Contributions

MS and YF collected the specimens and discussed and checked the final draft. MS photographed the specimens. YF conducted the genetic and morphological analysis and wrote the manuscript.

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